PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Molecular Genetics of Human Prion Diseases

John Collinge and Mark S. Palmer

Phil. Trans. R. Soc. Lond. B 1994 **343**, 371-378
doi: 10.1098/rstb.1994.0031

Molecular genetics of human prion diseases

JOHN COLLINGE AND MARK S. PALMER

Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, Imperial College, London W2 1PG, U.K.

SUMMARY

Human prion diseases occur in inherited, sporadic and acquired forms. The inherited forms are associated with coding mutations in the prion protein gene and the identification of one of these pathogenic mutations allows definitive diagnosis and has resulted in a widening of the previously recognized phenotypic spectrum of these diseases. Study of acquired prion disease provides evidence for genetic susceptibility to development of disease following treatment with contaminated pituitary hormones. Sporadic prion disease occurs predominately in individuals homozygous with respect to a common PrP polymorphism at residue 129. The identification of pathogenic PrP alleles and the role of the codon 129 PrP gene polymorphism in determining susceptibility to prion disease provides strong support for the idea that an abnormal isoform of PrP, PrP^{Sc}, is the principal constituent of the prion and that its propagation involves direct PrP–PrP interactions which occur most readily between identical PrP molecules.

1. INTRODUCTION

The prion diseases are a closely related group of neurodegenerative conditions which affect both humans and animals. They have previously been described as the sub-acute spongiform encephalopathies, slow virus diseases and transmissible dementias. The prototypic disease is scrapie, a naturally occurring disease affecting sheep and goats, which has been recognized in the U.K. for over 200 years (McGowan 1922). More recently recognized animal diseases include transmissible mink encephalopathy (Marsh *et al.* 1991), chronic wasting disease of mule deer and elk (Williams *et al.* 1980) and bovine spongiform encephalopathy (BSE) (Wells *et al.* 1987). The recently described feline spongiform encephalopathy of domestic cats (Wyatt *et al.* 1991) and spongiform encephalopathies of a number of zoo animals (Jeffrey *et al.* 1988; Kirkwood *et al.* 1990) are also now recognized as animal prion diseases. It is now clear that the epidemic of BSE among British cattle has resulted from the inclusion of dietary supplements prepared from the rendered carcasses of scrapie-infected sheep and, later, BSE-infected cows (Wilesmith *et al.* 1988). It seems likely that spongiform encephalopathies of other captive animals have also arisen from such dietary exposure to the transmissible agent. However, the mechanism of transmission of 'natural' sheep scrapie still remains unclear.

Traditionally, the human diseases have been subdivided into kuru, Creutzfeldt–Jakob disease and Gerstmann-Sträussler syndrome, and are all, like the animal diseases, transmissible to a range of experimental animals by intracerebral or other routes of inoculation (Gibbs *et al.* 1973). Kuru reached epidemic proportions amongst the Fore peoples of Papua New Guinea and was thought to be transmitted by ritualistic cannibalism (Alpers 1987). Other documented examples of case-to-case spread in humans has resulted from a number of iatrogenic routes. These include the use of inadequately sterilized intracerebral electrodes, dura mater and corneal grafting, and most recently treatment with human cadaveric derived pituitary growth hormone and gonadotrophin. Considerable concern has been raised that the inclusion of BSE-infected meat and more specifically offal in the production of human foodstuffs may result in the transmission of BSE to humans, although there is no evidence for this at present.

The spongiform encephalopathies both of animals and humans share common histopathological features. These include the classic triad of spongy degeneration (affecting any part of the cerebral grey matter), neuronal loss and the proliferation and hypertrophy of astrocytes (Beck & Daniel 1987). These changes are accompanied by accumulation in the brain of an abnormal, partially protease-resistant isoform of a host-encoded protein, prion protein, as amyloid (Prusiner 1987). A wide body of experimental evidence suggests that this abnormal, partly protease-resistant isoform of PrP is an essential, and may be the sole, constituent of the transmissible agent. Purified PrP fractions retain infectivity which is resistant to treatment with agents known to inactivate nucleic acids (Prusiner 1987).

The term 'Creutzfeldt–Jakob disease' (CJD) was first used by Spielmeyer in 1922, bringing together the original case report by Creutzfeldt in 1920 with subsequent cases described by Jakob. The term was used in subsequent years to cover a wide range of neurodegenerative conditions, and was not a very clearly defined diagnostic entity. In the late 1950s

Phil. Trans. R. Soc. Lond. B (1994) **343**, 371–378
Printed in Great Britain

Hadlow (Hadlow 1959) and also Klatzo and colleagues (Klatzo *et al.* 1959) pointed out the neuropathological similarity between scrapie, kuru and CJD. The successful transmission of kuru in 1966 and CJD in 1968 to the chimpanzee by Gadjusek and colleagues (Gajdusek *et al.* 1966; Gibbs *et al.* 1968) led to the emergence of the concept of the transmissible dementias. Experimental transmission work allowed diagnostic criteria for CJD to be reassessed and refined. CJD is currently recognized clinically by the occurrence of a rapidly progressive dementia with myoclonus which may be accompanied by pyramidal signs, cerebellar ataxia or extrapyramidal features. The clinical course is usually rapid and many cases die within three months of onset. The duration of illness in the vast majority of cases is less than 12 months. Routine investigations are generally unhelpful; in particular there is no evidence of an infective process occurring. The only useful investigation is the electroencephalogram which may show characteristic pseudoperiodic sharp wave activity (Cathala & Baron 1987). Neuroimaging reveals only varying degrees of cerebral atrophy. Around 10% of CJD cases present atypically with a longer duration of illness (Brown *et al.* 1984). The incidence of CJD seems remarkably uniform throughout the world where systematic epidemiological surveys have been performed. The incidence is around 0.5 to 1 case per million population per annum, and has an apparently random distribution related only to the local population density (Brown *et al.* 1987). There are however exceptions to this in the form of three ethnogeographic clusters of CJD (see later). Despite its transmissibility, CJD therefore does not epidemiologically resemble an infectious disease. There is no evidence of case to case spread with the exception of the rare iatrogenic cases discussed previously. In addition there is no relationship of the incidence of CJD with local scrapie prevalence. However around 15% of cases of CJD occur in a familial context.

Gerstmann–Sträussler syndrome (GSS) was first described in 1928 by Gerstmann in a single patient (Gerstmann 1928). A more detailed report of this individual and seven other members from the same Austrian family followed in 1936 by Gerstmann, Sträussler and Scheinker (Gerstmann *et al.* 1936). The condition is also referred to as Gerstmann–Sträussler–Scheinker disease. The classic description of GSS is of a chronic cerebellar ataxia with dementia occurring later in a much more prolonged clinical course. The mean duration of illness is around 5 years. The pathological hallmark of GSS is the presence of multicentric amyloid plaques distributed throughout the brain. Neuronal loss, spongiform change and white matter loss is seen in most cases (Masters *et al.* 1981). GSS was transmitted to experimental animals in 1981 (Masters *et al.* 1981). GSS usually occurs in a familial context and like familial CJD the disease segregation suggests an autosomal dominant pattern of inheritance.

Partial amino acid sequencing of the N-terminal of PrP isolated from the brains of affected hamsters and subsequent screening of cDNA libraries led to the realisation that the prion protein was encoded by a host chromosomal gene and not, as had previously been thought, by a viral genome (Chesebro *et al.* 1985; Oesch *et al.* 1985; Basler *et al.* 1986). The PrP gene in humans maps to the short arm of chromosome 20 (Sparkes *et al.* 1986; Robakis *et al.* 1986). Because all the spongiform encephalopathies are associated with accumulation in the brain of an abnormal isoform of PrP, the PrP gene was clearly a strong candidate gene for genetic linkage studies in GSS and familial forms of CJD. Hsiao *et al.* in 1989 first reported genetic linkage between the PrP gene and GSS in two families, demonstrating GSS to be an autosomal dominant inherited condition in addition to being transmissible to experimental animals by inoculation with brain homogenate (Hsiao *et al.* 1989). The polymorphism identified by Hsiao and colleagues was not an anonymous DNA marker but a missense mutation at codon 102 of the PrP gene open reading frame which results in a proline to leucine substitution in the mutant protein. Proline 102 is very highly conserved; all mammalian PrP genes sequenced to date encode proline at the corresponding codon. This mutation has now been confirmed in a large number of GSS kindreds in the U.K., U.S.A., Germany, Italy and Japan (Doh ura *et al.* 1989; Goldgaber *et al.* 1989; Speer *et al.* 1991; Kretzschmar *et al.* 1992) and indeed in the original Austrian family reported by Gerstmann (Kretzschmar *et al.* 1991). This mutation has not however been seen in several hundred controls. It therefore seemed highly likely on statistical grounds that this leucine substitution was a pathogenic mutation causing GSS in these families. Another mutation had previously been reported in the PrP gene consisting of 144 base pair (b.p.) insertion in a U.K. family with Creutzfeldt–Jakob disease (Owen *et al.* 1989). Since the report of these first two mutations a large number of other point mutations and other insertional mutations have now been reported in the PrP gene in affected or at risk individuals in families with these inherited diseases. Formal demonstration that the leucine 102 mutation was pathogenic was provided by Hsiao *et al.* (1990), who demonstrated that transgenic mice encoding multiple copies of a prion protein transgene encoding leucine at the corresponding murine codon spontaneously developed spongiform neurodegeneration. It has further been demonstrated that brain homogenates from such spontaneously sick transgenic mice can transmit the disease to wild-type animals (Hsiao *et al.* 1992). This production of a transmissible disease by mutation of a single amino acid in the prion protein argues strongly in favour of the prion protein, or rather an abnormal isoform of this protein, being the principal constituent of the transmissible agent or prion.

The availability of direct gene markers for the inherited diseases has enabled study of the phenotypic range of these disorders. Using DNA markers it has been possible to demonstrate remarkable phenotypic heterogeneity within families and also to identify some previously unrecognized prion diseases. Initial observations in this regard were made in the kindred with the 144 b.p. insertion in the PrP gene (Owen *et al.*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1989). The diagnosis of familial CJD in this kindred was based on an individual who died in the 1940s with both a clinical and neuropathological diagnosis of CJD. Indeed, the typicality of the histopathological features of this case have led to its inclusion illustrating CJD pathology in Greenfield's Neuropathology Textbook. The clinical course was said to be characteristic of CJD with a six-month duration. However another affected individual from this family had a duration of illness of over four years and at autopsy only mild and subtle spongiform changes, insufficient for a morphological diagnosis of CJD, were seen in the hippocampus. An additional family member has had the illness for over 12 years. Therefore a range of illness from that of classical sub-acute CJD to a GSS-like picture exceeding the longest previously published clinical duration for GSS can co-exist in the same family with the same genetic mutation. Additionally the histological features can vary from gross to minimal. For this reason it was argued that other GSS/CJD-type illnesses might present atypically and mimic other neurodegenerative conditions. This led to screening of neurodegenerative cases and the identification of families with mutations in the PrP gene who were not thought on clinical ground to be suffering from spongiform encephalopathies. The first family to be detected in this way using PrP gene analysis had been thought clinically to be suffering from familial Alzheimer's disease (Collinge *et al.* 1989). Further screening of over 100 cases revealed a further four families with an identical PrP gene insertion (Owen *et al.* 1991) and subsequent genealogical investigation demonstrated that all these cases form part of a single large kindred in the southeast of England (Poulter *et al.* 1992). Previous clinical diagnosis in these families had included familial Alzheimer's disease, Huntington's disease, Pick's disease and familial presenile dementia (Collinge *et al.* 1992). Of particular interest in this regard was a family which had carried previous clinical diagnoses of Huntington's disease and familial Alzheimer's disease. The clinical features of one member of this family were most unusual. The patient had a long-standing personality disorder characterized by irritability, aggression, antisocial behaviour and hypersexuality. He became increasingly aggressive in his early 20s and developed loss of balance and dysarthria. By his late 20s he had developed obvious intellectual decline, memory loss and a profound dyspraxia. At age 30 he had negligible abilities on verbal tasks, severe memory impairment, gross ataxia and episodes of clonic activity later developing generalized seizures. By age 36 he was permanently hypertonic, unable to talk and died of bronchial pneumonia. At autopsy there was mild cerebral atrophy but a remarkable absence of histological features of CJD, GSS or Alzheimer's disease. This finding raised the possibility that prion diseases were under-diagnosed, as perhaps 10% of patients dying with dementia who undergo autopsy do not have sufficient histological findings to reach a diagnosis (Collinge *et al.* 1990). Therefore diagnostic terms such as CJD, GSS and spongiform encephalopathy are becoming less useful with the demonstration that CJD and GSS form part of a wider spectrum of disease and can coexist in the same family with the same mutation. The histological features regarded as being characteristic of these disorders are not invariably present. Since an aberrant form of the prion protein and its gene play a key role in the aetiology of these conditions, prion disease, which can then be subdivided into acquired, sporadic and inherited forms, seems a more suitable terminology.

2. INHERITED PRION DISEASES

At least 18 pathogenic mutations are now recognized within the PrP gene leading to the inherited prion diseases. The identification of one of these mutations allows molecular diagnosis in an affected case. In addition such work has led to further insights into the phenotypic range of these disorders which now extends well beyond that previously realized. Pathogenic mutations reported to date in the human PrP gene are of two groups: (i) point mutations within coding sequence resulting in amino-acid substitutions (or in one case, in a stop codon, with production of a truncated protein) in PrP at residues 102, 105, 117, 145, 178, 180, 198, 200, 210, 217 and 232; and (ii) insertional mutations encoding 2, 4, 5, 6, 7, 8 or 9 additional copies of an octapeptide repeat that is present in a tandem array of five copies in the normal protein.

While cases within the 144 b.p. insertion pedigree clinically resembled Alzheimer's disease a number of types of inherited prion disease have now been identified that not only clinically but also in many respects histologically resemble Alzheimer's disease. Inherited prion disease (PrP 216 b.p. insertion) shows no spongiform encephalopathy histologically, but marked plaque deposition (Duchen *et al.* 1993). In the hippocampus there are neuritic plaques positive for both β-amyloid protein and tau. In the cerebellum and basal ganglia the plaques are PrP positive. Neurofibrillary tangles were also seen. Recently reported is a point mutation resulting in an amber mutant at codon 145 (Kitamoto *et al.* 1993). This case phenotypically appeared like Alzheimer's disease, both at the clinical and neuropathological level. The plaques were however PrP positive. In the case of inherited prion disease (PrP serine 198) and inherited prion disease (PrP arginine 217) as well as prominent PrP-positive plaques there are large numbers of neurofibrillary tangles which are antigenically and morphologically indistinguishable from those seen in Alzheimer's disease (Hsiao *et al.* 1992).

Since the first report of a six octarepeat insertional mutation in PrP a whole family of insertional mutations are now recognized (Goldfarb *et al.* 1991*a*; Owen *et al.* 1992). Insertions have not been described in normal individuals (other than individuals at risk in affected families) with the exception of a four octarepeat insertion reported in an individual who died at age 63 of hepatic cirrhosis (Goldfarb *et al.* 1991*a*). Recently, we have identified a patient with a classical phenotype of CJD (both clinically and histologically) with a four octarepeat insertion. Although this mutation differs from that published by Goldfarb *et al.*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

at the nucleotide level, the same protein is encoded (J. Collinge & M. S. Palmer, unpublished results). This may represent incomplete penetrance of this mutation.

Of particular interest has been the finding of a mutation at codon 178 in several families with what is described as fatal familial insomnia (Medori *et al.* 1992*a*,*b*). This disease is characterized by progressive untreatable insomnia and autonomic dysfunction. This disease has now been reclassified as one of the inherited prion diseases and, interestingly, carries the same mutation as that seen in several families from Northern Europe with a phenotype like Creutzfeldt–Jakob disease (Goldfarb *et al.* 1991*b*).

Although CJD usually occurs as a sporadic disease without clustering of cases, there are three well-documented ethnogeographic clusters of CJD that were an apparent exception to this random distribution of cases, amongst Libyan Jews, in an area of Slovakia and in Chile. All three clusters are now known to be familial and associated with the codon 200 mutation (Goldfarb *et al.* 1990*a*,*b*; Hsiao *et al.* 1991; Brown *et al.* 1992*a*). Cases of inherited prion disease (PrP lysine 200) have now been identified in the U.K., and at least one of these has no apparent genealogical link to the known clusters suggesting a separate U.K. focus of this disease (Collinge *et al.* 1993).

The availability of such direct gene tests for the inherited forms of these diseases allows not only unequivocal diagnosis but also presymptomatic testing of unaffected but at risk family members (Collinge *et al.* 1991*b*) and also opens up the potential for antenatal testing. In some families it may also be possible to determine whether a gene carrier will have an early or late onset of disease by codon 129 genotyping (discussed later). Most of the inherited prion diseases described to date show full penetrance although information is still very limited on a large number of these mutations. A particular exception is inherited prion disease (PrP leucine 200) in which elderly unaffected gene carriers have been identified. The genetic counselling situation in many respects resembles that seen in Huntington's disease and it is important to appreciate that performing PrP gene analysis for diagnostic purposes may have important consequences for other family members and that such issues should be discussed with the family prior to testing.

In addition to pathogenic PrP mutations, a number of coding and non-coding polymorphisms have been described. A common protein polymorphism at residue 129 is known to be important in genetic susceptibility to prion diseases (see below). Deletions of a single octarepeat element have been reported both in patients and normal controls (Laplanche *et al.* 1990; Diedrich *et al.* 1992). We studied 482 patients with a range of neurodegenerative illnesses including acquired, sporadic and inherited prion diseases and 255 unrelated caucasian normal controls and found five deletions in the patient group (0.52% of alleles) and two deletions in the controls (0.39% of alleles). However, only one of the deletions was in a definite CJD case. This difference in frequency was not statistically significant and we estimated a population frequency of deletions in caucasians as 1% of the population. We identified three different deletions at the nucleotide level although all encode the same protein (Palmer *et al.* 1993). There is no evidence therefore that deletions are associated with disease. It remains to be established if their co-occurence in a family with an inherited prion disease may produce a modification of the phenotype.

3. IATROGENIC PRION DISEASE

Although prion diseases can be transmitted to experimental animals by intracerebral inoculation with brain homogenates, it is important to appreciate that they are not contagious conditions in humans. Documented case-to-case spread in humans has only occurred as a result of ritualistic cannibalism in the case of kuru or in Western societies as a result of accidental inoculation with prions. Such iatrogenic routes include the use of inadequately sterilized intracerebral electrodes, dura mater and corneal grafting, and from the use of human cadaveric pituitary-derived growth hormone or gonadotrophin. These cases are extremely rare. It is of interest that cases arising from intracerebral or optic inoculation manifest clinically with a phenotype reminiscent of classical Creutzfeldt–Jakob disease with a rapidly progressive dementia, while those resulting from a peripheral route of inoculation with the agent present usually as a progressive cerebellar syndrome, reminiscent of kuru.

We investigated the possibility that genetic susceptibility may have a part to play in the occurrence of iatrogenic CJD. We chose to look at a group of individuals who had unfortunately developed CJD following treatment with human pituitary derived growth hormone. At the time of study six cases had arisen in the U.K., 1908 individuals having been treated with hormone derived from human pituitary. In addition a single case had arisen in the U.K. as a result of treatment with pituitary derived gonadotrophin in Australia. It was known that no single batch of hormone spanned all the cases and that such batches were prepared from pools of very large numbers of glands (up to 3000). It therefore seemed possible, if not likely, that most of the patients treated with growth hormone were exposed to the same amount of the transmissible agent. We hypothesized therefore that the ones that went on to get CJD may represent a genetically susceptible population. Known pathogenic mutations were excluded from all cases but the distribution of genotypes with respect to a common polymorphism in the PrP was quite different from the population frequencies. There is a common polymorphism in human PrP at residue 129, methionine being encoded in 63% of alleles and valine in 37% of alleles in Caucasians. This gives a genotype frequency of 37% Met/Met 21% Met/Val and 12% Val/Val (as estimated using a sample population of 106 normal controls). However, of the seven iatrogenic CJD samples analyzed, four were Val/Val, two were Met/Val and only one Met/Met (Collinge *et al.*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1991*a*). Although the number of cases analysed was necessarily small (but included all U.K. pituitary hormone related iatrogenic CJD at the time of the study) the data suggested that there was genetic susceptibility to exogenous prion infection. Individuals exposed to contaminated pituitary hormones who are valine homozygotes may be at increased risk (albeit still a small risk) of developing CJD. Similar results have now been reported in U.S. pituitary hormone cases (Brown *et al.* 1992*b*). The implications of this data with respect to a general model of prion propagation are discussed later. It is possible that iatrogenic CJD cases arising from intracerebral or optic inoculation with prions may not show such an effect as the intracerebral route of inoculation is known to be very much more efficient than a peripheral route. We have genotyped only one such case, related to dura mater grafting, and the genotype was Met/Met. It will be of interest to see if cases of kuru occur predominantly in a Val homozygous genotype.

4. SPORADIC PRION DISEASE

Most cases of human prion disease currently recognized occur as sporadic CJD. By definition there will not be a family history in such cases. However, we have on occasion found pathogenic mutations in apparently sporadic cases. With a late onset disease, family history may not be immediately apparent. The parent with the mutation may of course have died from another illness prior to the development of neurodegeneration. Additionally, non-paternity accounts for a number of such cases. With these exceptions however most sporadic CJD is of unknown aetiology. Our finding of the importance of genotype at codon 129 in genetic susceptibility to iatrogenic prion disease led us to genotype a sample of sporadic CJD cases. If sporadic CJD were being acquired from some environmental source of prions, for instance from scrapie infected sheep or another animal source there may also be an over representation of the valine 129 homozygous genotype. We initially studied 22 well-characterized sporadic CJD cases and found that all but a single case were homozygous either to methionine or valine at codon 129 (Palmer *et al.* 1991). There was no significant excess of valine homozygotes. Although 51% of the normal caucasian population are heterozygotes, nearly all sporadic CJD appears to occur in homozygotes. This finding has now been replicated on a larger series in conjunction with the U.K. CJD Surveillance Unit (J. Collinge, M. S. Palmer and R. G. Will, unpublished). The implications of this finding will be discussed below.

5. A MODEL OF PRION PROPAGATION

In 1990 Prusiner and colleagues demonstrated that the species barrier to transmission of prion disease from hamsters to mice could be overcome by overexpression of normal hamster prion protein in transgenic mice (Prusiner *et al.* 1990). Although prion diseases can be transmitted between mammalian species by inoculation, in practice it is difficult to do so, and typically transmission occurs in only a small proportion of inoculated animals and then only after prolonged incubation periods. When such transgenic mice were challenged with mouse derived prions, the infectivity they produced was fully pathogenic for mice but not hamsters, whereas on challenge with hamster derived prions they produced prions fully pathogenic to hamsters but not mice. This finding has been interpreted as implying that a direct interaction between PrP molecules occurs at some stage in the process of prion propagation and that the efficiency of such interaction is dependent on the degree of sequence homology between the interacting species. As there are a number of amino acid differences between murine and hamster PrP interaction between the two forms would be less favoured than interaction between PrP molecules with the same primary structure. Such work has led to the idea that replication of prions may occur by the abnormal isoform of PrP, PrP^{Sc}, interacting directly, either by dimerization or more complex interaction, with the normal cellular isoform, PrP^{C}, and catalysing the conversion of PrP^{C} to PrP^{Sc} (Prusiner *et al.* 1990; Weissmann 1991). Such an effect could then lead to a chain reaction of conversion leading to the progressive accumulation of the abnormal, and partly protease resistant isoform. This could then produce disease either by progressive loss of PrP function or alternatively by cellular damage resulting from accumulation of the protease resistant isoform. Clearly, the implication is that the pathogenic mutations in the prion protein result in the production of a protein that may convert spontaneously to PrP^{Sc} in individuals with inherited prion diseases. Once produced, PrP^{Sc} will then produce the same catalytic chain reaction as hypothesized in cases where PrP^{Sc} is inoculated. Such a model provides an explanation of how a disease can be simultaneously inherited and transmissible. The finding that nearly all sporadic CJD occurs in homozygotes with respect to a common and apparently innocent protein polymorphism lends strong support to such a mechanism (Palmer *et al.* 1991). Again prion protein interaction would occur most favourably in individuals with two identical copies of the prion protein. Heterozygotes producing two different proteins would be somewhat protected, as if by an internal 'species barrier'. It is possible that the occasional individuals heterozygous at codon 129 who do develop sporadic CJD have a more prolonged illness although more detailed studies are required to investigate this further (Collinge *et al.* 1991). Further evidence for this model of prion replication is provided by the observation that in inherited prion disease (PrP 144 b.p. insertion) the age at onset of the disease is 1–2 decades later in individuals heterozygous at codon 129 than homozygotes at codon 129 (Baker *et al.* 1991). This effect has now been observed in additional inherited prion disease kindreds.

We argued that PrP valine 129 and PrP methionine 129 will differ slightly in their propensity to convert to PrP^{Sc}. The excess of PrP valine 129 homozygotes

amongst human pituitary hormone related cases suggests that PrP valine 129 may be the more susceptible (Collinge *et al.* 1991*a*). Valine homozygotes would therefore be the most susceptible to prion diseases following environmental exposure to prions while heterozygotes would be partially protected. However, the genotype of the inoculum clearly will be relevant and it can not be assumed that val 129 homozygotes would also have increased susceptibility to animal prions compared to met 129 homozygotes. However, heterozygotes should still be protected according to such a model. Such ideas are supported by the recent finding (Büeler *et al.* 1993) that mice heterozygous for an ablated PrP allele, and which have approximately 50% of normal PrP expression, are highly resistant to prion disease following experimental inoculation, having extremely prolonged incubation times and also prolonged durations of illness.

6. CONCLUSIONS

The human prion diseases can be subdivided into inherited, sporadic and acquired forms. All currently recognized inherited prion diseases are caused by coding mutations in the PrP gene. It is not yet clear whether all of these 18 types of inherited prion disease will prove to be transmissible to experimental animals. It is possible that some of them may only be transmissible to transgenic animals expressing an homologous PrP. Some may have a different disease mechanism not producing infective prions but rather represent prion protein diseases. Sporadic prion disease occurs mainly in homozygotes with respect to a common PrP polymorphism. These cases could conceivably arise by somatic mutation producing one of the pathogenic PrP mutations, resulting in production of prions in the cell (or clone of cells) with the mutation, then leading to a progressive disease. Alternatively spontaneous conversion of PrP^{C} to PrP^{Sc} may occur as a rare stochastic event in the wild-type protein. Recent work indicating that transgenic mice with extremely high levels of hamster PrP^{C} production spontaneously develop a prion disease raises the additional possibility that some cases of inherited or sporadic prion disease may be caused by overexpression of PrP^{C}, either resulting from polymorphism in the PrP gene regulatory elements, the involvement of other genes, or environmental factors. Acquired prion diseases are caused direct inoculation with prions either by ritualistic cannibalism or iatrogenic routes. At least in the case of pituitary hormone related cases where low dose, peripheral inoculation resulted in the illness, genetic susceptibility is an important factor. It remains to be seen if the dietary exposure to bovine prions will result in transmission to humans. Recent success in removing the species barrier between hamsters and mice in transgenic mice raises the possibility that it may be possible to generate transgenic mice expressing human PrP variants that will be highly susceptible to human prion diseases, and in which the effectiveness of the bovine–human species barrier can be experimentally addressed.

REFERENCES

Alpers, M.P. 1987 Epidemiology and clinical aspects of kuru. In *Prions: novel infectious pathogens causing scrapie and Creutzfeldt–Jakob disease* (ed. S. B. Prusiner & M. P. McKinley), pp. 451–465. San Diego: Academic Press.

Baker, H.F., Poulter, M., Crow, T.J. *et al.* 1991 Amino acid polymorphism in human prion protein and age at death in inherited prion disease. *Lancet* **337**, 1286.

Basler, K., Oesch, B., Scott, M. *et al.* 1986 Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* **46**, 417–428.

Beck, E. & Daniel, P.M. 1987 Neuropathology of transmissible spongiform encephalopathies. In *Prions: novel infectious pathogens causing scrapie and Creutzfeldt–Jakob disease* (ed. S. B. Prusiner & M. P. McKinley), pp. 331–385. San Diego: Academic Press.

Brown, P., Rodgers-Johnson, P., Cathala, F., Gibbs, C.J. Jr & Gajdusek, D.C. 1984 Creutzfeldt–Jakob disease of long duration: clinicopathological characteristics, transmissibility, and differential diagnosis. *Ann. Neurol.* **16**, 295–304.

Brown, P., Cathala, F., Raubertas, R.F., Gajdusek, D.C. & Castaigne, P. 1987 The epidemiology of Creutzfeldt–Jakob disease: conclusion of a 15-year investigation in France and review of the world literature. *Neurology* **37**, 895–904.

Brown, P., Galvez, S., Goldfarb, L.G. *et al.* 1992*a* Familial Creutzfeldt–Jakob disease in Chile is associated with the codon 200 mutation of the PRNP amyloid precursor gene on chromosome 20. *J. neurol. Sci.* **112**, 65–67.

Brown, P., Preece, M.A. & Will, R.G. 1992*b* "Friendly fire" in medicine: hormones, homografts, and Creutzfeldt–Jakob disease. *Lancet* **340**, 24–27.

Büehler, H., Aguzzi, A., Sailer, A. *et al.* 1993 Mice devoid of PrP are resistant to scrapie. *Cell* **73,** 1339–1347.

Cathala, F. & Baron, H. 1987 Clinical aspects of Creutzfeldt–Jakob disease. In *Prions: novel infectious pathogens causing scrapie and Creutzfeldt–Jakob disease* (ed. S. B. Prusiner & M. P. McKinley), pp. 467–509. San Diego: Academic Press.

Chesebro, B., Race, R., Wehrly, K. *et al.* 1985 Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. *Nature, Lond.* **315**, 331–333.

Collinge, J., Harding, A.E., Owen, F. *et al.* 1989 Diagnosis of Gerstmann–Sträussler syndrome in familial dementia with prion protein gene analysis. *Lancet* **2**, 15–17.

Collinge, J., Owen, F., Poulter, M. *et al.* 1990 Prion dementia without characteristic pathology. *Lancet* **336**, 7–9.

Collinge, J., Palmer, M.S. & Dryden, A.J. 1991*a* Genetic predisposition to iatrogenic Creutzfeldt–Jakob disease. *Lancet* **337**, 1441–1442.

Collinge, J., Poulter, M., Davis, M.B. *et al.* 1991*b* Presymptomatic detection or exclusion of prion protein gene defects in families with inherited prion diseases. *Am. J. Hum. Genet.* **49**, 1351–1354.

Collinge, J., Brown, J., Hardy, J. *et al.* 1992 Inherited prion disease with 144 base pair gene insertion: II: Clinical and pathological features. *Brain* **115**, 687–710.

Collinge, J., Palmer, M.S., Campbell, T.A., Sidle, K.C.L., Carroll, D. & Harding, A.E. 1993 Inherited prion disease (PrP lysine 200) in Britain: two case reports. *Br. med. J.* **306**, 301–302.

Collinge, J. & Palmer, M.S. 1991 CJD discrepancy. *Nature, Lond.* **353**, 802.

Diedrich, J.F., Knopman, D.S., List, J.F. *et al.* 1992 Deletion in the prion protein gene in a demented patient. *Hum. molec. Genet.* **1**, 443–444.

Doh-ura, K., Tateishi, J., Sasaki, H., Kitamoto, T. &

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Sakaki, Y. 1989 Pro–leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann–Sträussler syndrome. *Biochem. biophys. Res. Commun.* **163**, 974–979.

Duchen, L.W., Poulter, M. & Harding, A.E. 1993 Dementia associated with a 216 base pair insertion in the prion protein gene. Clinical and neuropathological features. *Brain* **116**, 555–567.

Gajdusek, D.C., Gibbs, C.J. & Alpers, M.P. 1966 Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature, Lond.* **209**, 794–796.

Gerstmann, J. 1928 Über ein noch nicht beschriebenes Reflexphänomen bei einer Erkrankung des zerebellaren Systems. *Wien. Med. Wochenschr.* **78**, 906–908.

Gerstmann, J., Sträussler, E. & Scheinker, I. 1936 Über eine eigenartige hereditär-familiäre Erkrankung des Zentralnervensystems. Zugleich ein Beitrag zur Frage des vorzeitigen lakalen Alterns. *Z. Neurol.* **154**, 736–762.

Gibbs, C.J., Gajdusek, D.C., Asher, D.M. *et al.* 1968 Creutzfeldt–Jakob disease (spongiform encephalopathy): transmission to the chimpanzee. *Science, Wash.* **161**, 388–389.

Gibbs, C.J. & Gajdusek, D.C. 1973 Experimental subacute spongiform encephalopathies in primates and other animals. *Science, Wash.* **182**, 67–68.

Goldfarb, L.G., Korczyn, A.D., Brown, P., Chapman, J. & Gajdusek, D.C. 1990*a* Mutation in codon 200 of scrapie amyloid precursor gene linked to Creutzfeldt–Jakob disease in Sephardic Jews of Libyan and non-Libyan origin. *Lancet* **336**, 637–638.

Goldfarb, L.G., Mitrova, E., Brown, P., Toh, B.K. & Gajdusek, D.C. 1990*b* Mutation in codon 200 of scrapie amyloid protein gene in two clusters of Creutzfeldt–Jakob disease in Slovakia. *Lancet* **336**, 514–515.

Goldfarb, L.G., Brown, P., McCombie, W.R. *et al.* 1991*a* Transmissible familial Creutzfeldt–Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the *PRNP* gene. *Proc. natn. Acad. Sci. U.S.A.* **88**, 10926–10930.

Goldfarb, L.G., Haltia, M., Brown, P. *et al.* 1991*b* New mutation in scrapie amyloid precursor gene (at codon 178) in Finnish Creutzfeldt–Jakob kindred. *Lancet* **337**, 425.

Goldgaber, D., Goldfarb, L.G., Brown, P. *et al.* 1989 Mutations in familial Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker's syndrome. *Expl. Neurol.* **106**, 204–206.

Hadlow, W.J. 1959 Scrapie and kuru. *Lancet* ii, 289–290.

Hsiao, K., Baker, H.F., Crow, T.J. *et al.* 1989 Linkage of a prion protein missense variant to Gerstmann–Sträussler syndrome. *Nature, Lond.* **338**, 342–345.

Hsiao, K., Meiner, Z., Kahana, E. *et al.* 1991 Mutation of the prion protein in Libyan Jews with Creutzfeldt–Jakob disease. *N. Engl. J. Med.* **324**, 1091–1097.

Hsiao, K., Dlouhy, S.R., Farlow, M.R. *et al.* 1992 Mutant prion proteins in Gerstmann–Sträussler–Sheinker disease with neurofibrillary tangles. *NatureGenetics* **1**, 68–71.

Hsiao, K.K., Scott, M., Foster, D., Groth, D.F., DeArmond, S.J. & Prusiner, S.B. 1990 Spontaneous neurodegeneration in transgenic mice with mutant prion protein. *Science, Wash.* **250**, 1587–1590.

Hsiao, K.K., Groth, D., Scott, M. *et al.* 1992 Genetic and transgenetic studies of prion proteins in Gerstmann–Sträussler–Scheinker disease. In *Prion diseases of humans and animals* (ed. S. B. Prusiner, J. Collinge, J. Powell & B. Anderton), pp. 120–128. London: Ellis Horwood.

Jeffrey, M. & Wells, G.A. 1988 Spongiform encephalopathy in a nyala (*Tragelaphus angasi*). *Vet. Pathol.* **25**, 398–399.

Kirkwood, J.K., Wells, G.A., Wilesmith, J.W., Cunningham, A.A. & Jackson, S.I. 1990 Spongiform encephalopathy in an arabian oryx (*Oryx leucoryx*) and a greater kudu (*Tragelaphus strepsiceros*). *Vet. Rec.* **127**, 418–420.

Kitamoto, T., Iizuka, R. & Tateishi, J. 1993 An amber mutation of prion protein in Gerstmann–Sträussler syndrome with mutant PrP plaques. *Biochem. biophys. Res. Commun.* **192**, 525–531.

Klatzo, I., Gajdusek, D.C. & Zigas, V. 1959 Pathology of kuru. *Lab. Invest.* **8**, 799–847.

Kretzschmar, H.A., Honold, G., Seitelberger, F. *et al.* 1991 Prion protein mutation in family first reported by Gerstmann, Sträussler, and Scheinker. *Lancet* **337**, 1160.

Kretzschmar, H.A., Kufer, P., Riethmuller, G., DeArmond, S., Prusiner, S.B. & Schiffer, D. 1992 Prion protein mutation at codon 102 in an Italian family with Gerstmann–Sträussler–Scheinker syndrome. *Neurology* **42**, 809–810.

Laplanche, J.L., Chatelain, J., Launay, J.M., Gazengel, C. & Vidaud, M. 1990 Deletion in prion protein gene in a Moroccan family. *Nucl. Acids. Res.* **18**, 6745.

Marsh, R.F., Bessen, R.A., Lehmann, S. & Hartsough, G.R. 1991 Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy. *J. gen. Virol.* **72**, 589–594.

Masters, C.L., Gajdusek, D.C. & Gibbs, C.J. Jr 1981 Creutzfeldt–Jakob disease virus isolations from the Gerstmann–Sträussler syndrome with an analysis of the various forms of amyloid plaque deposition in the virus-induced spongiform encephalopathies. *Brain* **104**, 559–588.

McGowan, J.P. 1922 Scrapie in sheep. *Scott. J. Agric.* **5**, 365–375.

Medori, R., Montagna, P., Tritschler, H.J. *et al.* 1992*a* Fatal familial insomnia: A second kindred with mutation of prion protein gene at codon 178. *Neurology* **42**, 669–670.

Medori, R., Tritschler, H.J., LeBlanc, A. *et al.* 1992*b* Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N. Engl. J. Med.* **326**, 444–449.

Oesch, B., Westaway, D., Walchli, M. *et al.* 1985 A cellular gene encodes scrapie PrP 27–30 protein. *Cell* **40**, 735–746.

Owen, F., Poulter, M., Lofthouse, R. *et al.* 1989 Insertion in prion protein gene in familial Creutzfeldt–Jakob disease. *Lancet* **1**, 51–52.

Owen, F., Poulter, M., Collinge, J. *et al.* 1991 Insertions in the prion protein gene in atypical dementias. *Expl. Neurol.* **112**, 240–242.

Owen, F., Poulter, M., Collinge, J. *et al.* 1992 A dementing illness associated with a novel insertion in the prion protein gene. *Molec. Brain Res.* **13**, 155–157.

Palmer, M.S., Dryden, A.J., Hughes, J.T. & Collinge, J. 1991 Homozygous prion protein genotype predisposes to sporadic Creutzfeldt–Jakob disease. *Nature, Lond.* **352**, 340–342.

Palmer, M.S., Mahal, S.P., Campbell, T.A. *et al.* 1993 Deletions in the prion protein gene are not associated with CJD. *Hum. molec. Genet.* **2**, 541–544.

Poulter, M., Baker, H.F., Frith, C.D. *et al.* 1992 Inherited prion disease with 144 base pair gene insertion: I: Genealogical and molecular studies. *Brain* **115**, 675–685.

Prusiner, S.B. 1987 Prions and neurodegenerative diseases. *N. Engl. J. Med.* **317**, 1571–1581.

Prusiner, S.B., Scott, M., Foster, D. *et al.* 1990 Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* **63**, 673–686.

Robakis, N.K., Devine Gage, E.A., Jenkins, E.C. *et al.* 1986 Localization of a human gene homologous to the PrP gene on the p arm of chromosome 20 and detection of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PrP-related antigens in normal human brain. *Biochem. biophys. Res. Commun.* **140**, 758–765.

Sparkes, R.S., Simon, M., Cohn, V.H. *et al.* 1986 Assignment of the human and mouse prion protein genes to homologous chromosomes. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7358–7362.

Speer, M.C., Goldgaber, D., Goldfarb, L.G., Roses, A.D. & Pericak-Vance, M.A. 1991 Support of linkage of Gerstmann–Sträussler–Scheinker syndrome to the prion protein gene on chromosome 20p12-pter. *Genomics* **9**, 366–368.

Weissmann, C. 1991 Spongiform encephalopathies. The prion's progress. *Nature, Lond.* **349**, 569–571.

Wells, G.A.H., Scott, A.C., Johnson, C.T. *et al.* 1987 A novel progressive spongiform encephalopathy in cattle. *Vet. Rec.* **121**(18), 419–420.

Wilesmith, J.W., Wells, G.A., Cranwell, M.P. & Ryan, J.B. 1988 Bovine spongiform encephalopathy: epidemiological studies. *Vet. Rec.* **123**, 638–644.

Williams, E.S. & Young, S. 1980 Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *J. Wildl. Dis.* **16**, 89–98.

Wyatt, J.M., Pearson, G.R., Smerdon, T.N., Gruffydd-Jones, T.J., Wells, G.A.H. & Wilesmith, J.W. 1991 Naturally occurring scrapie-like spongiform encephalopathy in five domestic cats. *Vet. Rec.* **129**, 233–236.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES